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13. ABSTRACT (Maximum 200 Words)

Numerous microarray experiments have identified many genes that have altered expression during the progression of breast cancer. Dauer development in C. elegans is controlled primarily by a TGFbeta-related pathway, so this system is ideal for studying this signaling pathway. Our hypothesis is that genes that are identified by genetics as important regulators of dauer development will be important regulators of breast cancer progression. We identified genes that are implicated in TGFbeta signaling in C. elegans and genes that are implicated in breast cancer progression using microarray data. We tested the function of these genes in TGFbeta signaling in C. elegans by RNAi gene knockout. We hoped that the use of a relatively simple system to rapidly learn more about the function of a tumorigenic pathway in breast cancer, will provide a basic foundation for understanding how this pathway acts in the more complex context of breast cancer. We found that the assay for determinining whether a particular gene affected dauer formation in C. elegans had unexpected problems, and we spent most of our effort solving these problems, and testing a small set of genes for roles in C. elegans TGFbeta signaling.

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Table of Contents

INTRODUCTION:

Transforming Growth Factorβ (TGFβ) signaling plays an important roles in establishment and maintenance of breast cancer, but the pathway both promotes and inhibits tumor growth, depending on the cell's interaction with other cells and signaling pathways. TGFβ is usually considered to be a growth inhibitor in early stages of breast cancer. For example, human primary breast cancer tumors and cell lines often show decreases in TGFβ receptor expression and/or responsiveness to the growth inhibitory effects of TGFβ (Wakefield et al, 2001). TGFβ signaling plays a causative role in mammary tumor suppression in a mouse model (Bottinger et al, 1997, Pierce et al 1995). But the effects of TGFβ are more complex and depend substantially of the environment and internal state of a cell. TGFβ also stimulated the growth of the breast cancer line MCF in defined medium, yet inhibited in serum containing medium (Croxtall et al, 1992). TGFβ signaling is often constitutively activated in metastases, suggesting that TGFβ promotes metastasis, perhaps via its ability to remodel the extracellular matrix (Derynck et al, 2001). TGFβ receptor activation causes metastasis in a mouse model; blockage of TGFβ receptor signaling in the breast cancer cell line 4T1 decreased its ability to metastasize by 10-fold (McEarchern et al 2001).

Insulin-like growth factor (IGF) signaling also plays a critical role in breast cancer growth, but little is known about how TGFβ and IGF signaling interact in breast cancer. Mice overexpressing IGF-I or IGF-II in the mammary gland developed mammary adenocarcinoma at very high rates (Bates, et al 1995; Hadsell et al. 2000). Some interaction between IGF and TGFβ signaling has been seen. For example, IGF-I and TGFβ synergistically regulate the activity of estrogen synthetic enzymes in breast cancer cell lines (Wong et al 2001). Yoneda et al. (2001) isolated bone seeking and brain seeking clones from the breast cancer cell line MDA-231. The parental cells and brain seeking cells showed TGFβ inhibition of anchorage-independent growth in soft agar, but no effect of IGF-I. The bone seeking cells had simultaneous changes in response to both growth factors: IGF-I stimulated anchorage independent growth, but TGFβ no longer had an effect. While these examples suggest a connection between IGF and TGFβ, the mechanistic nature of the connection remains obscure.

Microarray analysis of TGFβ and IGF target genes and genes with altered expression in breast cancer cells is identifying many genes with potentially important roles in breast cancer. Large sets of genes have been identified, which have expression changes correlated with cancer type, stage, clinical prognosis, IGF or TGFβ pathway activation, or mutations in tumor

suppressors or oncogenes (Clement et al., 2000; Hedenfalk, et al 2001; Martin et al, 2000; Nacht et al, 1999; Oh, et al 2002; Porter et al., 2001; Sorlie et al, 2001; Su et al. 2001; van't Veer et al, 2002; West et al., 2001; Zajchowski et al., 2001). The number of genes with changes in expression correlated with breast cancer progression is over 5000. Because TGFβ and IGF play an important role in breast cancer, many of these changes in expression are probably caused by perturbation of these two signaling pathways. However, we do not know which genes are regulated by which pathways, nor do we know which genes are the most important causes of cancer progression. Some of these genes have a known role in breast cancer. Others have a plausible connection, such as ABCC11, which is an ABC transporter that may be involved in drug resistance. Most interesting are the "new" genes--those that do not have a known role in breast cancer. Some of these genes, such as CALML3 (calmodulin-like 3) and WNT4 have known biochemical or biological functions, but the relationship of these functions to breast cancer is unknown. Other genes are orphans, for which a function has not yet been identified. Any of these "new" genes might have an important role in TGFβ or IGF signaling, and in breast cancer; however, some of the genes are probably upregulated as a consequence of other changes, but are not participating in breast cancer progression. And in most cases, we do not know which genes are responding to perturbations of TGFβ and IGF, and which are responding to other oncogenes or tumor suppressors.

Dauer formation is a developmental event in C. elegans (Riddle 1997). This process is controlled in a fundamental way by TGFβ and IGF-I signaling; the identification of these two pathways in unbiased genetic screens for dauer formation mutants suggests that these pathways are *the major means* by which the dauer decision is regulated (Patterson et al., 1997, Paradis et al, 1999, Paradis and Ruvkun, 1998, Ogg et al., 1997, Kimura et al., Inoue and Thomas, 2000, Georgi et al., 1990). Both pathways control a common endpoint, dauer formation, and the effect of the genes on the endpoint is synergistic, so integration of signals from the two pathways is an important feature of dauer regulation.

Our proposal was based on this unparalleled opportunity to study TGFβ and IGF-I signaling. The process is simple (relative to breast cancer progression), which simplifies the experiments and their interpretation, which in turn, promotes rapid progress in understanding the problem. The process is controlled in a fundamental way by TGFβ and IGF-I signaling. The existence of null mutants in most of the genes allows the pathways to be manipulated in a clean way—we can work with animals that are completely lacking individual components of one or both pathways. Both pathways control a common endpoint, dauer formation, and the effect of the genes on the endpoint is synergistic, so integration of signals from the two pathways is an important feature of dauer regulation. This fact means that we can learn

much about the mechanism by which these two types of signaling pathways are integrated.

BODY:

Our approved statement of work for this grant included three major tasks:

Task 1. Identify targets for functional tests.
a. The most relevant breast cancer microarray experiments will be identified, data downloaded and genes matched to C. elegans homologs
b. Targets will be selected on the basis of strong regulation of expression, high similarity between human and C. elegans, and putative likelihood of regulatory role in breast cancer.

We identified breast cancer microarray experiments for which we expected to find the most interesting targets, based on our evaluation of the clinical significance of the results (Martin et al, 2000; Oh, et al 2002; Sorlie et al, 2001; van't Veer et al, 2002; West et al., 2001; Zajchowski et al., 2001). From these, we extracted a small number of targets for initial assays of dauer formation; these targets are shown in Table 1 of the appendix. We intended to use these targets to test and optimize the assay, with the further intention of extracting more targets as needed.

Task 2. Initial test of targets, months 2-7.
a. Three to five hundred of the most promising targets will be assayed for effects on dauer formation.
b. Targets with promising results in initial screen will be retested to verify effects

In attempting to perform this task, we had technical difficulties. Our assay required that we use RNAi to test the function of genes in dauer formation, and we chose the feeding assay (Kamath et al. 2000) Our protocol is to allow adults to lay 100-200 eggs on a plate with the appropriate RNAi bacteria. We used a *daf-4* (type II receptor) partial loss of function mutant to provide maximum sensitivity. For positive controls, we used RNAi with *daf-3* (suppresses the *daf-4* partial loss of function) and *daf-4* (enhances the *daf-4* partial loss of function). Initially, we used empty vector as a negative control, but as described below, this was problematic. After three days at the appropriate temperature, we counted dauers and non-dauers. For each experiment shown in Table 2, we did three plates in triplicate of each genotype. In attempting to use this assay, we had two major technical problems.

The first technical problem was that our negative control did not work. The standard negative control is to compare animals in which RNAi of the gene of interest is induced to identically handled samples in which an empty vector is used in place of the clone of the gene of interest. We found that this negative control gave consistently low rates of dauer formation. After some effort to solve this problem, we settled on using a set of C. elegans genes with no detectable effect on dauer formation as a negative control.

The second, and much more troublesome, technical difficulty was that the assays were highly variable. We used known TGFβ pathway genes as positive controls: one gene (*daf-4*) that would be expected to increase dauer formation, and another (*daf-3*) that would be expected to decrease dauer formation. Initially, these controls did not work reliably, but we optimized the protocol and got these controls working reasonably well. Once we had the positive controls working well, we still saw large differences in results from experiment to experiment with the experimental genes. We devoted most of our time to further optimizing the protocol to get results that we believed were satisfactory.

In the process of optimizing our protocol, we chose to use the genes shown in Table 2 of the appendix, rather than the genes identified in task 1, because these RNAi clones had been shown to function in other assays. We felt that having clones of known quality was important given the difficulty with the assay.

In the end, we found that the variation in the assay was such that significant results were seen only after 10 or more experiments. This number of experiments is prohibitively difficult. The design of our proposal depended on having a quick and straightforward assay, a goal that we were unable to achieve.

As a consequence of these technical difficulties, we were not able to test as many genes as we proposed for task 2, and we were unable to proceed to task 3. Because we tested only a few genes, and these genes were not the genes identified in task 1, we are unable to comment on our original hypothesis that genes identified as important in dauer formation are important in cancer progression.

KEY RESEARCH ACCOMPLISHMENTS:

**Developed assay for testing the effect of RNAi on dauer formation

**Tested a small number of genes for effects on dauer formation

REPORTABLE OUTCOMES:

Abstracts

Control of aging and developmental arrest by TGFβ and insulin pathways during C. elegans diapause
Tao Liu, Manjing Pan, Garth Patterson
2004 East Coast C. elegans Meeting abstract

CONCLUSIONS:

We attempted to develop an assay for the study of breast cancer related genes in C. elegans. For technical reasons, our assay was inadequate to achieve our goal.

References

Bates P, Fisher R, Ward A, Richardson L, Hill DJ, Graham CF. 1995. Mammary cancer in transgenic mice expressing insulin-like growth factor II (IGF-II). *British Journal of Cancer* **72**:1189-93.

Bottinger EP, Jakubczak JL, Haines DC, Bagnall K, Wakefield LM. 1997. Transgenic mice overexpressing a dominant-negative mutant type II transforming growth factorbeta receptor show enhanced tumorigenesis in the mammary gland and lung in response to the carcinogen 7,12-dimethylbenz-[®]-anthracene. *Cancer Res.* **57**:5564-70.

Clement JH, Marr N, Meissner A, Schwalbe M, Sebald W, Kliche KO, Hoffken K, Wolfl S. 2000. Bone morphogenetic protein 2 (BMP-2) induces sequential changes of Id gene expression in the breast cancer cell line MCF-7. *J Cancer Res Clin Oncol.* **126**:271-9.

Croxtall JD, Jamil A, Ayub M, Colletta AA, White JO. 1992. TGF-beta stimulation of endometrial and breast-cancer cell growth. *Int J Cancer.* **50**:822-7.

Derynck R, Akhurst RJ, Balmain A. 2001. TGF-β signaling in tumor suppression and cancer progression. *Nature Genetics.* **29**:117-9.

Hadsell DL, Murphy KL, Bonnette SG, Reece N, Laucirica R, Rosen JM. 2000. Cooperative interaction between mutant p53 and des(1 -3)IGF-I accelerates mammary tumorigenesis. *Oncogene* **19**:889-98.

Hedenfald I, Duggan D, Chen Y, Radmacher M, Bittner M, Simon R,

Meltzer P, Gusterson B, Esteller M, Kallioniemi O, Wilfond B, Borg A, Trent J. 2001. Gene expression profiles in hereditary breast cancer. *The New England Journal of Medicine*. **344**:539-548.

Inoue T, Thomas JH. 2000. Targets of TGF-beta signaling in Caenorhabditis elegans dauer formation. *Dev Biol.* **217**:192-204.

Kamath R, Martinez-Campos M, Zipperlen P, Fraser A, Ahringer J. 2000. Effectiveness of specific RNA-mediated interference through ingested double-stranded RNA in *Caenorhabditis elegans. Genome Research* **2:**1-10.

Kimura KD, Tissenbaum HA, Liu Y, Ruvkun G. 1997. daf-2, an insulin receptor-like gene that regulates longevity and diapause in Caenorhabditis elegans. *Science* **277**:942-6.

Martin KJ, Kritzman BM, Price LM, Koh B, Kwan CP, Zhang X, Mackay A, O'Hare MJ, Kaelin CM, Mutter GL, Pardee AB, Sager R. 2000. Linking Gene Expression Patterns to Therapeutic Groups in Breast Cancer. *Cancer Research.* **60**:2232-8.

McEarchern JA, Kobie JJ, Mack V, Wu RS, Meade-Tollin L, Arteaga CL, Dumont N, Besselsen D, Seftor E, Hendrix MJC, Katsanis E, Akporaiye ET. 2001. Invasion and metastasis of a mammary tumor involves TGF-β signaling. *Int. J. Cancer.* **91**:76–82.

Morris JZ, Tissenbaum HA, Ruvkun G. 1996. A phosphatidylinositol-3-OH kinase family member regulating longevity and diapause in *Caenorhabditis elegans*. *Nature* **382**:536-9.

Nacht, MA, Ferguson T, Zhang W, Petroziello JM, Cook BP, Gao YH, Maguire S, Riley D, Coppola G, Landes GM, Madden SL, Sukumar S. 1999. Combining Serial Analysis of Gene Expression and Array Technologies to Identify Genes Differentially Expressed in Breast Cancer. *Cancer Research.* **59**:5464-70.

Oh JS, Kucaby JE, Bushel PR, Martin K, Bennett L, Collins J, DiAugustine RP, Barrett JC, Afsharix CA, Dunny SE. 2002. Insulin-Like Growth Factor-1 Inscribes a Gene Expression Profile for Angiogenic Factors and Cancer Progression in Breast Epithelial Cells. *Neoplasia* . **4**:204-17.

Ogg S, Paradis S, Gottlieb S, Patterson GI, Lee L, Tissenbaum HA, Ruvkun G.. 1997. The Fork head transcription factor DAF-16 transduces insulin-like metabolic and longevity signals in C. elegans. *Nature* **389**:994-9.

Paradis S, Ailion M, Toker A, Thomas JH, Ruvkun G. 1999. A PDK1 homolog is necessary and sufficient to transduce AGE-1 PI-3 kinase signals that regulate diapause in *C. elegans*. *Genes Dev.* **13**:1438-52.

Paradis S, Ruvkun G. 1998. Caenorhabditis elegans Akt/PKB transduces insulin receptor-like signals from AGE-1 PI3 kinase to the DAF-16 transcription factor. *Genes Dev.* **12**:2488-98.

Patterson GI, Koweek A, Wong A, Liu Y, Ruvkun G. 1997. The DAF-3 Smad protein antagonizes TGF-β-related receptor signaling in the*Caenorhabditis elegans* dauer pathway. *Genes and Development* **11**:2679-90.

Pierce DF, Jr., Gorska AE, Chytil A, Meise KS, Page DL, Coffey RJ, Jr., Moses HL. 1995. Mammary tumor suppression by transforming growth factor beta1 transgene expression. *Proc. Natl. Acad. Sci. USA.* **92**:4254-8.

Porter DA, Krop IE, Nasser S, Sgroi D, Kaelin CM, Marks JR, Riggins G, Polyak K. 2001. A SAGE (Serial Analysis of Gene Expression) View of Breast Tumor Progression. *Cancer Research.* **61**:5697-702.

Ren P, *et al.* 1996. Control of C. elegans Larval Development by Neuronal Expression of a TGF-b homologue. *Science* **274**:1389-1391.

Riddle DL. 1997. Genetic and environmental regulation of dauer larva development. In *C. elegans II* (eds. Riddle DL, Blumenthal, T., Meyer, B.J. & Priess, J.R.) 739-68. Cold Spring Harbor Press.

Sørlie T, Perou CM, Tibshirani R, Aas T, Geisler S, Johnsen H, Hastie T, Eisen MB, van de Rijn M, Jeffrey SS, Thorsen T, Quist H, Matese JC, Brown PO, Botstein D, Lønning PE, Børresen-Dale AL. 2001. Gene expression patterns of breast carcinomas distinguish tumor subclasses with clinical implications. *Proc Natl Acad Sci USA* **98**:10869-74.

Su AI, Welsh JB, Sapinoso LM, Kern SG, Dimitrov P, Lapp H, Schultz PG, Powell SM, Moskaluk CA, Frierson HF, Jr., Hampton GM. 2001. Molecular Classification of Human Carcinomas by Use of Gene Expression Signatures. *Cancer Research.* **61**:7388-93.

van 't Veer LJ, Dai H, van de Vijver MJ, He YD, Hart AA, Mao M, Peterse HL, van der Kooy K, Marton MJ, Witteveen AT, Schreiber GJ, Kerkhoven RM, Roberts C, Linsley PS, Bernards R, Friend SH. 2002. Gene expression profiling predicts clinical outcome of breast cancer. *Nature*. **415**:530-6.

Wakefield LM, Piek E, Bottinger EP. 2001. TGF-β Signaling in Mammary Gland Development and Tumorigenesis. *J Mammary Gland Biol Neoplasia.* **6**:67-82.

Wong SF, Reimann K, Lai LC. 2001. Effect of TGFβ1, IGF-1 and IGF-2 on cell growth and oestrogen metabolism in human breast cancer cell lines. *Pathology* **33**:454-9.

Yoneda T, Williams PJ, Hiraga T, Niewolna M, Nishimura R. 2001. A bone-seeking clone exhibits different biological properties from the MDA-MB-231 parental human breast cancer cells and a brain-seeking clone in vivo and in vitro. *J Bone Miner Res.* **16**:1486-95.

Zajchowski DA, Bartholdi MF, Gong Y, Webster L, Liu HL, Munishkin A, Beauheim C, Harvey S, Ethier SP, Johnson PH. 2001. Identification of Gene Expression Profiles That Predict the Aggressive Behavior of Breast Cancer Cells. *Cancer Res.* **61**:5168-78.

Appendix

Table 1. Genes regulated in C. elegans TGFβ mutants and in breast cancer.

Human Gene	How implicated in breast cancer	Biochemical or Biological Role	C. elegans homolog	Expression in C. elegans TGFβ mutants
AGR2-- Anterior gradient 2 (Xenopus) homolog	coexpressed w/estrogen re-ceptor in breast cancer cells	unknown	F49H12. E	2.5 fold up
RCN1; RCN Reticulocalbin 1	expression correlated with invasive potential	unknown, resides in endoplasmic reticulum, binds calcium	M03F4.7	2 fold up
ANP32D-member of the pp32 protein family	some pp32s are oncogenes, others tumor suppressors	histone acetylase inhibition	T19H12. 2	2.5 fold down
NDRG1-N-myc down-stream regulated gene 1	downregulated in breast cancer	regulates proliferation and differentiation by unknown mechanism	ZK1073. 1	2 fold down
DRIM—Down regulated in metastasis	downregulated in metastatic breast cancers	unknown	F18C5.3	2.5 fold down
EIF3S3, Eukaryotic translation initiation	Amplified and overexpressed in BC	regulation of protein synthesis	C41D11. 2	2 fold down
aa487428	Expression correlated with breast cancer type	unknown	M02B7.2	2 fold down
flj10948	Expression correlated with breast cancer type	Similar to AUH, which binds to the 3' untranslated region of oncogene mRNAs	F11A3.1,	3 fold down
flj13758	Expression correlated with breast cancer type	unknown	F49e12.1 0	2 fold down
crabp1-cellular retinoic acid binding protein 1	Expression correlated with breast cancer type	may influence the intracellular level of free retinoic acid	T22G5.2, T22G5.6	4 fold down 2 fold up

Table 2. RNAi knockdown assays.

Gene name	function	Dauer expr[1]	Log odds ratio[2]	T test p value[3]	Binomial p value[4]	Log odds ratio of individual experiments[5]			
						>0.8	0 to 0.8	<-0.8	0 to -0.8
DAF-3	control		-4.53	7E-06	8E-06	0	0	17	0
F33D11.1	LIM domain	2.5	-1.12	0.07	0.05	1	1	6	2
T07D10.4	C type lectin	6.3	-1.02	0.01	0.03	1	2	9	2
L4440	control		-0.95	0.01	0.07	1	4	7	5
C17H1.2	novel	3.5	-0.82	0.01	0.01	0	1	5	4
K07A1.7	novel	3.4	-0.66	0.07	0.16	0	2	5	0
T03F1.6	novel	2.4	-0.65	0.24	0.31	1	2	3	0
C35A5.8	novel	1.0	-0.58	0.33	0.27	1	2	2	2
B0414.3	histone H1 and H5	-2.6	-0.56	0.31	0.31	0	3	3	0
F09C3.1	ras GAP	-2.7	-0.43	0.25	0.09	4	1	5	5
K03B8.7	novel	18.9	-0.42	0.7	0.38	1	0	2	0
R09B3.2	RNA recognition	-3.5	-0.41	0.4	0.23	0	2	2	2
R11A5.6	novel	-2.3	-0.33	0.25	0.16	0	1	1	3
T27F6.2	C-type lectin	6.1	-0.17	0.61	0.16	1	6	1	3
M01E11.5	Cold shock DNA binding	-2.3	-0.15	0.77	0.15	6	3	3	3
T23B3.2	UPF0057	2.1	-0.13	0.37	0.31	0	2	0	3
C17H1.7	novel	9.5	0.03	0.93	0.17	3	6	3	4
C33H5.4	kinesin-like	-1.5	0.33	0.23	0.09	4	3	1	1
C33H5.5	novel		0.45	0.11	0.13	4	5	1	3
H16D19.2	Lectin C-type	4.3	0.45	0.41	0.21	4	3	3	3
K09A11.4	p450	2.1	0.49	0.81	0.38	1	1	1	0
C34B2.4	LIM domain	6.1	0.54	0.29	0.21	5	1	3	4
C26C6.4	DUF271	4.3	0.87	0.02	0.03	4	5	0	2
C35E7.9	major sperm protein	3.8	0.97	0.01	0.01	7	5	1	1
F46F11.2	Cold shock DNA binding	-3.5	1.17	0.05	0.05	8	2	0	3
DAF-4	control		1.69	9E-04	1E-04	12	4	1	0

[1] This is the fold change in expression comparing dauer animals to non-dauer animals.

[2] The logs odd ratio is defined as:

$$\frac{\text{number of dauer animals in experimental}}{\text{number of non-dauers in negative control}} * \frac{\text{number of dauers in negative control}}{\text{number of non-dauers in experimental}}$$

A positive log odds ratio indicates that the experimental RNAi favored dauer formation (interfered with TGFbeta signaling), and a negative value the converse.

[3] This p value uses the log odds ratio in a t test against the null hypothesis of no effect, i.e. log odds ratio of zero

[4] This p value is non-parametric and compares the number of experiments with positive and negative log odds ratios with the null hypothesis of no effect (equal numbers of experiments with positive and negative values.

[5] These four columns show the numbers of independent experiments performed for each gene. Ranges of log odds ratios, and the number of experiment in each range, are shown.